**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | | |
|---|---|---|
| In re Application of: | Gregory T. Bleck, et al. | |
| Serial No.: | 10/759,315 | Group No.: 1633 |
| Filed: | 1/16/04 | Examiner: Riggins |

Entitled: **PRODUCTION OF HOST CELLS CONTAINING MULTIPLE INTEGRATING VECTORS BY SERIAL TRANSDUCTION**

# DECLARATION OF DR. GREGORY BLECK

EFS WEB FILING
Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

I, Dr. Gregory Bleck, state as follows:

1. My present position is Senior Director, Cell Line Engineering, Gala Biotech, a Catalent Pharma Solutions Company.

2. I am an inventor of the above referenced patent application.

3. It is my understanding that the Examiner has argued that one of skill in the art would have known to increase the production of a desired protein by increasing the number of viral integrations and would have known to increase the number of viral integrations by increasing the MOI.

4. This is not true. The art teaches away from producing cell lines by using a high MOI's, such as an MOI of 100, and also teaches away from the production of cell lines with more than 20 intregrated vectors per cell. Coffin et al., Development and Applications of Retroviral Vectors, Chapter 9 in Retroviruses, 1997, p. 437-473, which was cited by the previous Examiner on Form PTO-829 mailed November 17, 2005, teaches at page 463, column 1 that: "Insertional mutagenesis by retroviral vectors is often cited as a safety concern. This issue has been raised because proviral insertion can cause the inactivation of tumor suppressor genes or the activation of oncogenes." Furthermore, references such as Arai et al., Virology 260:109-115 (1999), which was cited by the previous Examiner on Form PTO-829 mailed November 17, 2005, specifically teach away from the current claims. Arai et al. state:

> When a 3Y1 was transduced with the pseudotyped vector at an m.o.i. of 100, a significant proportion of the cell population became detached from the plate within two days. Since apoptotic cells were detected from among these cells at 1 day after transduction by means of the TUNEL method (data not shown), proviral integration with a very high copy number seems to cause cell death. While we did not address the reason for this induction of apoptosis, a major factor could be that the multiple integration causes insertional mutagenesis in essential genes. The toxicity of overproduced LacZ was not the major reason, because LacZ activity observed in 3Y1 transduced at m.o.i. 100 at 1 day after the transduction was less than that detected in 3Y1 transduced at m.o.i. 30 at 3 days after the transduction, and the latter cells showed only marginal signs of apoptosis (data not shown).

Thus, Arai teaches that "proviral integration with a very high copy number seems to cause cell death." Upon reading reading Arai, one of skill in the art would be "discouraged" from using the claimed multiplicity of infection and copy insert number to obtain cells for the production of a secreted protein.

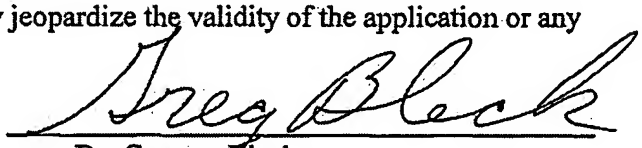
5. The Examiner attempts to distinguish Coffin et al. by arguing that it is noted that Coffin refers to in vivo use of retroviral vectors in human and animals and not to in vitro use as presently claimed. This is not a valid distinction. A person of skill in the art, reading Coffin et al., would be discouraged from using transduction conditions that lead to high numbers of integrations and insertional mutagenesis. Such concerns would apply in vivo or in vitro.

6. The Examiner admits that Arai et al. teaches that proviral integration with a high copy number seems to cause cell death. The Examiner attempts to distinguish Arai et al. by arguing that Arai et al. do not teach what a very high copy number means. A person of skill in the art, reading Arai et al., would understand Arai et al. to teach away from using conditions that could lead to insertional mutagenesis and cell death. The person of skill in the art understands that conditions that would result in more than 15 integrations fall within this category and thus be discouraged from exceeding the conditions taught by Arai. Thus, one of skill in the art would not have known to use MOIs between 30 and 100 to obtain more than 15 integrations and would not have known to use routine experimentation to determine what MOIs result in 20 to 100 integrations. In fact, a person of skill in the art, reading Arai et al., would have been discourages from such experiments because Arai teaches that insertional mutagenesis and cell death would result.

7. The Examiner's reliance on Taruscio et al. as teaching the meaning of high copy number is inappropriate. Taruscio et al. addresses the copy number of endogenous retroviruses that have integrated into the genome over millions of years, and perhaps been duplicated by chromosomal rearrangement over that extreme time period. This is a different context than the exposure of cultured cells to concentrated levels of retroviral vectors in vitro. Arai et al. and Coffin et al. address exactly the conditions at issue, i.e., transduction of cells with concentrated retroviral vectors, and conclude that such conditions cause insertional mutagenesis and cell death. One of skill in the art is discouraged from using such conditions. The prior art clearly warns of the risks associated using conditions that lead to greater than 15 integrations per cell.

8. I further declare that all statement made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: August 31, 2007



Dr. Gregory Bleck